



S/N 10/054,665

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: John F. Engelhardt et al.

Examiner: Unknown

Serial No.: 10/054,665

Group Art Unit: 1636

Filed: January 22, 2002

Docket: 875.007US2

Title: ADENO-ASSOCIATED VIRUS VECTORS

PRELIMINARY AMENDMENT

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Applicant's Representatives have reviewed the "Notice to File Missing Parts of Nonprovisional Application" mailed April 10, 2002. Please amend the above-identified patent application as follows:

In the Specification

Please enter the SEQUENCE LISTING into the specification.

Please make the paragraph substitutions indicated in the appendix entitled Clean Version of Amended Specification Paragraphs. The specific changes incorporated in the substitute paragraphs are shown in the following marked-up versions of the original paragraphs:

Please amend the paragraph beginning at line 1, page 10 of the specification as follows:

Figure 2. Structural analysis of rAAV circular intermediates in Hela cells (agarose gel, left; Southern blot, right). Circular rAAV intermediate clones isolated from AV.GFP3ori infected Hela cells were analyzed by diagnostic restriction digestion with AseI, SphI, and PstI together with Southern blotting against ITR, GFP, and Stuffer <sup>32</sup>P-labeled probes. In panel A, four clones representing the diversity of intermediates found (p190, p333, p280, and p345) gave a diagnostic PstI (P) restriction pattern (3kb and 1.7kb bands) consistent with a circular monomer or multimer intact genome. SphI (S) digestion demonstrated existence of a single ITR (p190), two ITRs in a head-to-tail orientation (p333 and p280), and three ITRs (p345) in isolated circular intermediates. The restriction pattern of pCisAV.GFP3ori (U; uncut, P; PstI cut, S; SphI cut) and 1 kb DNA ladder (L) are also given for comparison. One additional circular form (p340) was repetitively seen and had an unidentifiable structure which lacked intact ITR sequences. Circular concatamers were identified by partial digestion with AseI for clones p280 (dimer) and p333 (monomer) as is shown in Panel B. Sequence analysis (Panel C) of six clones with identical restriction patterns to p333 (Panel A) was performed using primers (indicated by arrows) juxtaposed to the partial p5 promoter (dotted line) and ITRs (solid line) (SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13). The top sequence